ANIMAL REPRODUCTION

Panel Manager – Dr. David Miller, University of Illinois
Program Director – Dr. Mark A. Mirando

The primary objective of this program area is to increase our knowledge of reproductive biology in agriculturally important animals with the goal of increasing reproductive efficiency. This program supports innovative research on: (1) factors controlling ovarian function including follicular development, ovulation, and corpus luteum formation and function, (2) factors controlling male reproduction, (3) gamete physiology, including oogenesis and spermatogenesis, gamete maturation, and mechanisms regulating gamete survival *in vivo* or *in vitro*, (4) mechanisms involved in placental function, and (5) parturition, postpartum interval to conception, and neonatal survival.

Because alterations in animal behavior and animal well-being may impair fertility, this program also encourages research on the mechanisms controlling animal responses to physical and biological stresses that impinge upon reproductive processes. Research should contribute to an understanding of the causes, consequences, and avoidance of stress, rather than merely describing the physiological effects of stress on reproductive efficiency.

2001-00955 Neuroendocrine Control of Shell Gland Contractility in the Domestic Hen
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Seed Grant; Grant 2001-35208-10842; $75,000; 2 Years

The ovulation-oviposition cycle of a laying domestic hen ranges from 24 to 28 hours. During this time, an egg spends approximately 20 hours in the shell gland where plumping, calcification, pigment formation and egg expulsion occur. Expulsion of the egg is facilitated by contraction of the shell gland myometrium. Shell gland contractions are initiated by arginine vasotocin (AVT), a hormone released from the pituitary gland, but prostaglandins may also be involved. The objective of this project is to establish how the shell gland is transformed from being quiescent during the majority of the ovulation-oviposition cycle to being a highly contractile organ at the time of an egg lay. Our working hypothesis, that the sensitivity of shell gland myometrium to the contractile actions of AVT and prostaglandins is regulated during the ovulation-oviposition cycle, will be tested by experiments described under two specific aims. First, we will characterize the stimulatory effect of AVT on prostaglandin biosynthesis by shell gland endometrial cells. Second, we will determine the expression levels of AVT receptors in the shell gland endometrium during the ovulation-oviposition cycle. This research will benefit U.S. agriculture by providing new information about hormones that influence egg production. Establishing the cellular events leading to increased shell gland contractility and expulsion of the egg should provide potential targets to control the time that the egg spends in the shell gland and thereby improve egg quality and eggshell strength. Achieving these goals should lead to an improved and more consistent product for consumers and increased profit for poultry producers.

2001-02162 Effect of Stress on Reproductive Function in Sheep
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Grant 2001-35203-11220; $235,000; 3 Years

Fertility is the product of an intricate and precisely regulated hormonal cascade that begins in the brain with the secretion of gonadotropin-releasing hormone (GnRH). This hypothalamic peptide, in turn, stimulates the secretion of the gonadotropic hormones, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), from the gonadotrope cells of the pituitary gland. The gonadotropic hormones stimulate gonadal development, leading to sperm production in males and ovulation in females. Stress reduces the fertility of domestic animals by disrupting the precise interplay among the regulatory

hormones, thereby, reducing follicle development and ovulation, increasing embryonic and fetal loss, extending the interval from calving or lambing to conception, and increasing the services required per conception. The economic impact of unavoidable stress can be significant. For example, heat stress reduces conception and embryonic viability by more than 80% in beef and dairy cows. Although the adverse effect of stress on animal fertility is well recognized, the physiological link between stress and reproduction is not defined precisely. Stress is commonly associated with increased secretion of cortisol from the adrenal gland. We propose to use sheep as a research model in studies designed to determine the role of cortisol in stress-induced infertility. We hypothesize that cortisol acts at pituitary loci to decrease gonadotrope responsiveness. We will use unique stimulatory and neutralizing antisera to define the role of the adrenal in stress-induced infertility. We anticipate that completion of the proposed studies will facilitate the development and implementation of management strategies and therapeutic interventions designed to lessen the magnitude and severity of the infertility induced by stress.

2001-02159 Use of GnRH-PAP for Chemical Sterilization

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Grant 2001-35203-10837; $250,000; 3 Years

The primary method for preventing reproduction in domestic livestock in the United States today is removal of testes or ovaries; this eliminates both fertility and sexual behavior. Although the procedure is routine in males, it is not without problems including infection and retarded growth rate for several days following the surgery. Unfortunately, the practical usefulness of this procedure is limited to males due to inaccessibility of the female gonads. However, it is equally desirable to eliminate fertility and sexual behavior in females. We are attempting to develop an injectable formulation that will permanently inhibit activity of the gonads in either sex of a variety of species. Our approach is to attach a protein (pokeweed antiviral protein, PAP) capable of killing cells to gonadotropin-releasing hormone (GnRH), the hormone responsible for controlling reproduction in both males and females of all mammals. Injection of this complex into an animal should cause death of the cells responsible for stimulating the testis or ovary without harming other cells in the body. There are several questions that must to be addressed before the practicability of this approach can be assessed. The experiments set forth in this grant address some of these questions. Namely, we plan to determine if the GnRH-PAP complex: (1) is sufficiently stable in the circulation of animals to inhibit the reproductive system; (2) if inhibition of the reproductive system is long-lasting (more than one year), and (3) is capable of inducing sterility in animals prior to puberty as well as in adult animals.

2001-02402 Identification of Expressed Polymorphisms and Imprinted Genes in Cattle

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Seed Grant; Grant 2001-35208-10875; $74,996; 2 Years

Cattle are economically important for U.S. agriculture and assisted reproductive technologies are essential to further genetic improvement of cattle. However, calves derived from these technologies, such as cloning and in vitro fertilization, are frequently associated with problems of abnormal fetal development, neonatal deaths and extra large calves (large calf syndrome, LCS). All of these increase cost to producers and hamper the wide application of these technologies. In mammals, genetic imprinting refers to the phenomenon that expression of certain genes depends on their parental origin. For example, only the sire's copy of the IGF-2 gene is expressed (mono-allelic expression). Most imprinted genes regulate fetal development. Interestingly, many of the LCS defects are similar to imprinting disruptions (bi-allelic expression of imprinted genes) in mice and human. Studies of bovine imprinting, however, have been hampered by the lack of information on expressed polymorphisms (small variations in genes) in cattle, which are essential for distinguishing the two parental copies of imprinted genes. Therefore, the aim of this seed grant is to search for polymorphisms in putative imprinted genes in cattle to reach our

long-term goal in understand the underlying causes for LCS. To achieve this, we plan to search for expressed polymorphisms in a selected group of potentially imprinted genes in the bovine. These genes are imprinted in mouse and/or human and disruption of these genes cause abnormal fetal development and/or fetal deaths. We also plan to determine if the above genes are imprinted in cattle. The identification of polymorphisms will lay a solid foundation for further studies on imprinting in cattle, which will provide potential solutions to LCS.

0102251 Gabaergic Control of Luteinizing Hormone Pulse Patterns

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Grant 2001-35203-10911; $180,000, 2 Years

The goal of this research is to improve the breeding efficiency of domestic animals by overcoming failure to breed (anestrus) due to factors such as season, stressors, or immaturity. The approach to be used is to develop a simple drug-based therapy that will act within the brain to induce secretion of gonadotropin-releasing hormone (GnRH) from the brain and luteinizing hormone (LH) from the pituitary gland. This, in turn, may induce onset of ovarian cycles. Emphasis will be placed on the gabaergic system, a major system in the brain that normally inhibits GnRH secretion. We have found that either localized microinjections into the brain or systemic infusion of the drug, baclofen, will elevate GnRH and LH secretion in rams. This drug acts by stimulating specific receptor sites named GABA-b receptors. The experiments in this project will determine: (1) if these drugs have similar effects in ewes, and (2) if blocking GABA-b receptors with drugs will prevent ovulation-inducing LH surges. The approach will be to infuse the drugs into specific sites in the brains of ewes in order to determine the effects on secretion of LH. In addition, the investigators will attempt to determine if the ovarian hormone, estrogen, regulates the number of GABA-b receptors in those parts of the brain that control GnRH release. We may also attempt to induce ovarian cycles in seasonally anestrous ewes by giving baclofen systemically.

2001-01351 21st Annual Meeting of the American Society for Reproductive Immunology

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Conference Grant; Grant 2001-35203-10646; $5,236; 1 Year

Studies of maternal immune responses to the pregnancy have practical implication in animal reproduction. Research outcome has been directly applied to decrease the infection in reproductive organs, increase fertility and develop a new technique to enhance fetal growth. It is critical for reproductive immunologists to be exposed to research by animal and veterinary scientists who work in reproductive immunology because the research questions studied by these scientists are often of general relevance to the reproductive immunologists. For this conference, the USDA-NRICGP supported the American Society for Reproductive Immunology (ASRI) to foster the continued development of large animal reproductive immunology by providing speaker travel expenses to the ASRI meeting. Funds supported three speakers whose research involves large animals. The meeting will be organized around six plenary sessions. The topics will include recent advances in reproductive immunology that have changed the understanding of maternal fetal immune tolerance and recurrent pregnancy losses, implantation failures and pregnancy complications, which are the ultimate goal of research to apply to the clinical setting. In addition, the roles of immune competent cells, regulatory molecules and autoimmune responses during pregnancy will be discussed. New frontier research in HIV and related viruses in the reproductive tract will be addressed also. The American Society for Reproductive Immunology has made great strides in recent years in increasing the involvement of large animal reproductive physiologists in its activities. The funding support of the USDA-NRICGP has been an important part of this effort.

2001-02336 Behavioral and Morphological Traits Associated with Fertility in Broiler Breeders

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Grant 2001-35203-10872; $245,000; 3 Years

A major component of the broiler industry success has been the improvement in efficiency reached due to genetic selection programs. The industry anticipates that genetic improvement will continue with birds growing faster and yielding more meat. However, it is recognized that this intense selection has been accompanied by reduced reproductive efficiency, to the point that male fertility may potentially become a limiting factor for growth of the broiler industry. The precise causes of this fertility reduction have not yet been determined, although it has been suggested to be related to the changes in male physical conditions and behavior, both of which may have impaired male vigor and mating ability. Studies of avian reproduction suggest that several behavioral and morphological characteristics could be used as reliable indicators of male quality. The goal of this project is to determine fundamental behavioral and morphological causes of reduced fertility in broiler breeders selected for high yield. Specific objectives of the study include: (1) quantify the impact of social dominance on male fertility; (2) determine the impact of male social status on semen quality and functionality, and (3) investigate the use of morphometric and behavioral traits as reliable indicators of high male fertility that can be incorporated into genetic selection protocols to improve flock fertility. The proposed project is unique in that it applies new concepts from behavioral theory to the improvement of fertility. This research has a multidisciplinary approach that will allow us to determine effects of behavioral and morphometric components at multiple levels, and includes an experiment in commercial conditions that will directly allow us to quantify the biologic and economic impact of the proposed research in the field.

2001-02255 Intrafollicular Role of Alpha-2-Macroglobulin in Regulation of Estradiol Production
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Grant 2001-35203-10910; $240,000; 3 Years

Alpha-2-macroglobulin is a large glycoprotein that acts as both a broad-spectrum proteinase inhibitor and growth factor binding protein. Interaction of alpha-2-macroglobulin with proteinases transforms alpha-2-macroglobulin's shape enabling it to bind receptors. The compelling rationale to study the intrafollicular role of alpha-2-macroglobulin is based on recent findings by the principal investigator showing that a positive correlation exists between intrafollicular amounts of alpha-2-macroglobulin and estradiol secretion during dominant follicle development in cows and that alpha-2-macroglobulin-induced increase in estradiol production by bovine granulosal cells isolated from dominant follicles is greater than any other known stimulator of estradiol secretion. These findings led to the hypothesis that increases in intrafollicular amounts of alpha-2-macroglobulin and transformation of alpha-2-macroglobulin by intrafollicular proteinases enhance capacity of granulosa cells to produce estradiol. To test this hypothesis, two aims are proposed: (1) determine the association between alterations in intrafollicular amounts of alpha-2-macroglobulin with estradiol during development of dominant follicles, and (2) establish whether conformational transformation of alpha-2-macroglobulin alters its capacity to enhance estradiol production by granulosa cells *in vitro*. To accomplish these aims, ultrasound will be used to identify stages of development of dominant follicles, and *in vivo* and *in vitro* approaches will be used to evaluate the role of alpha-2-macroglobulin during dominant follicle development. Because estradiol is a key hormone involved in synchronization of the physiological processes necessary for reproduction, understanding how estradiol production is regulated in dominant follicles may provide new information important for the design of better methods to control the estrous cycle and improve fertility.

2001-02346 Mechanisms and Gonadotropic Requirements for Bovine Follicular Selection and Maintenance
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Grant 2001-35203-10912; $101,606; 1 Year

Two major limitations to maximize reproductive efficiency in cattle include the failure of females to have an ovulatory estrus at the start of the breeding season (beef) or voluntary waiting period (dairy) and embryonic mortality. Although antral follicles are present on the ovaries of cattle regardless of the reproductive state, lack of an ovulatory estrus can be attributed to the failure of antral follicles to complete the maturation process culminating in the release of a fertilizable germ cell. Approximately 20% of the 36,000,000 total beef cows in the United States do not calve annually and approximately 12% of dairy cows are culled annually due to infertility. Thus, the birth of fewer calves alone results in a loss of $750,000,000 annually. Elucidation of endocrine, cellular and molecular mechanisms associated with follicular growth will provide the basis for development of therapeutic regimens to induce ovulatory follicles, improve methods for estrous synchronization and improve success for more uniform response to superovulation. Based on our previous work, there appear to be distinct changes in gene expression around the time of selection of the dominant follicle. The specific objective of this grant is to determine if abundance of mRNA for LH receptor in granulosa cells is a function of follicular age (*i.e.*, time dependent) or is hormonally induced.

2001-02275 Sex bias and Interferon Produced at Blastocyst
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Grant 2001-35203-10693; $275,000; 3 Years

Two surprising observations have been made on bovine embryos produced by *in vitro* maturation/*in vitro* fertilization procedures: (1) fewer female than male embryos cultured in presence of glucose, but not in its absence, make the transition from morula to embryonic blastocyst; (2) female embryos produce more interferon-tau than males once they reach the embryonic blastocyst stage of development. These *in vitro* phenomena may reflect events that occur *in vivo* and provide some plasticity in the ability of livestock to adjust the sex ratio of offspring born by selective loss of embryos of one sex relative to the other. We shall determine to what extent different culture conditions, particularly ones that are stressful, can modulate sex ratio and interferon-tau production of embryonic blastocyst. We shall assess whether the sex ratio among *in vivo*-produced blastocysts is close to 1:1 and whether the difference in interferon-tau production between the sexes is maintained *in vivo*. We shall also determine whether transfer of embryos after culture *in vitro* can be used to modulate sex ratios of offspring. Finally, we shall measure whether there is selective expression of a sub-group of interferon-tau genes by female blastocysts. These experiments provide two new criteria, sex ratio and interferon-tau production, for assessing optimal culture conditions for bovine embryos. They may also help explain how sex ratios can be modulated *in utero*, and how a bias in the sexes of calves born can either be prevented or exploited. Finally the work will provide further insights into how genes may become improperly regulated during embryo development.

2001-02334 Intrauterine Folate Binding Proteins during Pregnancy in Swine
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Grant 2001-35203-11241; $154,999; 2 Years

The number of piglets that can be maintained by the uterus during pregnancy is currently the most limiting factor to litter size, which is an important component in the profitability of swine production in the United States. The results of several experiments have suggested that red blood cell development by the piglet before birth is a factor that influences uterine capacity. The vitamin, folate, is known to be required for efficient red blood cell development, and results also suggest that the amount of folate

available to the developing piglet during pregnancy influences red blood cell development during gestation. Unfortunately, folate delivery to the developing piglet is not fully understood. We recently discovered two uterine folate binding proteins (FBPs) that likely play a central role in this process. It is not clear how these two FBP types interact to deliver folate during pregnancy. The experiments proposed in this grant will define when and where these proteins are made in the pig uterus, and how they interact with each other to accomplish folate delivery to the developing piglet. Using this information, it should be possible to improve the transfer of folate from the mother to the developing piglet, which would improve red blood cell development and oxygen carrying capacity of the developing piglet during pregnancy. This, in turn, would improve the survival of piglets within the uterus, and increase uterine capacity and litter size.

2001-02270 Regulation of Hypothalamic GnRH Secretion by Antigonadotropic Decapeptide
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Grant 2002-35203-11614, $200,000, 3 Years

Substantial economic gain for livestock producers could be realized by development of better means to enhance reproductive efficiency. Central to improving reproductive efficiency in domestic animals is understanding the regulatory processes controlling secretion of gonadotropin releasing hormone (GnRH). Secretion of GnRH has a profound effect on reproductive efficiency because it controls the normal occurrence of estrous cycles, as well as the duration of reproductive intervals associated with periods of anestrus and sexual maturation. Discovery of methods to activate GnRH secretion during periods of reproductive quiescence to initiate ovarian cyclicity would greatly enhance reproductive efficiency in domestic animals. To develop efficient means to manipulate GnRH secretion, a thorough understanding of mechanisms regulating GnRH secretion must first be developed. An important aspect of this proposal is our focus on a novel regulatory peptide, antigonadotropic decapeptide (AGD), that may greatly enhance our understanding of regulation of GnRH secretion. Our excitement over this new peptide comes from previous results that indicate that it is a potent inhibitor of GnRH secretion in the ewe. Our preliminary data indicates that this hormone participates as an autocrine feedback regulator of GnRH secretion. AGD also appears to regulate neurotransmitter input to GnRH neurons. Previous work demonstrate that AGD is more effective in inhibiting GnRH secretion during the anestrous season than during the breeding season in ewes. These observations led us to speculate that AGD may function as an autocrine and paracrine regulator of GnRH secretion, modulating activity of the reproductive system. Experiments will be conducted to examine the mechanisms by which AGD regulates hypothalamic release of GnRH. The long-range goals of these studies are to provide new basic knowledge that will be useful in developing methods to decrease intervals of reproductive quiescence in our meat- and milk-producing animals.

2001-02260 In vitro Spermatogenesis and Germ Cell Transfection in the Bull
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Grant 2001-35203-10836, $210,000, 3 Years

The long-term objective of this research is to develop an *in vitro* tissue culture system to study and exploit the mechanistic details of mammalian spermatogenesis. Efforts to improve sperm production in domestic animals through physiological manipulation of spermatogenesis have met with very little success. Progress has been limited because spermatogenesis *in vivo* is an extremely complex process and, though studied extensively in domestic animals, remains relatively poorly understood at the cellular level. Suitable long-term culture systems to investigate the regulation of spermatogenesis are needed but have not been available previously. We have developed a unique culture system in which haploid cells expressing spermatid-specific genes are generated from neonatal testis cells consistent with the timing of spermatogenesis *in vivo*. Preliminary studies indicate that the presumptive haploid cells produced in vitro

are competent to produce viable diploid embryos following microinjection into mature oocytes. Our immediate goal is to recapitulate completely the process of bovine spermatogenesis *in vitro*. We plan to characterize the histology, cytology, gene expression, and developmental competence of germ cells produced *in vitro* and evaluate conditions designed to improve the efficiency of the culture system. This information can then be used to address questions regarding the fundamental process of spermatogenesis *in vivo* and for practical applications including increased production of sperm for use in artificial insemination, treatment of male factor infertility such as maturation failure, and genetic modification of the male germ line.

2001-02169 Mechanisms Governing Movement of Sperm in the Oviduct

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Grant 2001-35203-11132, $229,000, 2 Years

Our goal is to learn how sperm movement is regulated within the bovine oviduct in order to bring about fertilization. Sperm are trapped just inside the entrance to the oviduct when a protein on the sperm head binds to a specific carbohydrate group on the epithelium lining the wall of the oviduct. Somehow, this interaction prolongs the fertility of sperm while holding them in reserve until ovulation. We have tentatively identified the sperm protein as PDC-109, a product of the seminal vesicles that adheres to sperm. We have also found that the carbohydrate that forms the oviductal binding site contains fucose. Our specific aims are: (1) to characterize the sperm protein, and (2) to identify the molecule containing the carbohydrate to which sperm bind. In aim 1, we will determine whether: (a) PDC-109 confers fucose-binding capacity on sperm; (b) PDC-109 binds sperm to the epithelium, and (c) PDC-109 is altered or removed by capacitation of sperm. We have shown that capacitated sperm no longer bind to oviductal epithelium. After identifying molecules in extracts of epithelium that contain fucose in aim 2, we will attach a label to PDC-109 and use it as a probe to identify its receptor. When a candidate is found, its amino acid sequence will be obtained to characterize the protein. Information arising from these studies may be used for improving sperm storage, increasing pregnancy rates after artificial insemination, and reducing the numbers of sperm required for artificial insemination, especially for the use of sexed semen.

2001-02328 IGF Actions and Signaling during Ovarian Follicle Maturation in Temperate Basses

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Grant 2001-35203-11131; $174,159; 3 Years

Although there are over 20,000 species of fishes, most species examined have failed to reproduce in captivity. Improved diagnostic tests to characterize reproductive status and hormone-based therapies to control reproduction are needed. Potential for new tests and tools based on insulin-like growth factors (IGFs) is great. IGFs have been shown to regulate proliferation and degeneration (apoptosis) of ovarian follicle cells and synthesis of sex steroid hormones by the ovarian follicle. IGFs also regulate aquisition of maturational competence by the follicle and oocyte maturation (meiosis). These are all phenomena that aquaculturists need to better diagnose and control. Most are affected by IGF-I in temperate basses (genus *Morone*) including striped bass and white bass, the parents of the hybrid striped bass which supports one of the fastest growing forms of fish farming in the United States. We propose to further characterize the reproductive effects and mechanism(s) of action of IGF-I in temperate basses. We will examine effects of IGF-I on apoptosis and components of maturational competence, including binding of the maturation-inducing steroid hormone to its oocyte receptor and coupling of the oocyte to follicle cells via signaling portals known as gap junctions. We will also use signaling pathway inhibitors and measure changes in signaling molecules or their activities in cultured follicles exposed to IGF-I. We aim to discover the signaling pathways by which IGF-I controls ovarian maturation. This information will lead to new diagnostic tools and procedures for controlling reproduction of cultured fishes.

2001-02257 Role of gap junctions in regulation of luteal function

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Standard Strengthening Award; Grant 2002-35203-11643; $170,000; 3 Years

The long term goal of this project is to study mammalian reproductive biology focusing on the investigation of reproductive function in farm animals. The specific objectives of this project are to evaluate: (1) the role of cellular interactions in regulation of hormones secretion by ovarian luteal cells and (2) the ontogeny of expression of several genes that encode proteins (connexins) mediating contact-dependent cellular interactions in ovarian tissues. Sheep will be used in the proposed studies because they provide a good model for ruminant species, including the cow, and are widely used in agricultural and biomedical research. Because it is the primary source of progesterone (the pro-gestational hormone), the ovary is responsible for maintaining cyclic reproductive function and pregnancy. It has been well documented that cell-to-cell communication is a fundamental biological process that is necessary to maintain a healthy state within tissues, and it is also important in normal growth, development and differentiation of cells and tissues. Abnormal intercellular communication leads to cellular dysfunction, such as uncontrolled tissue proliferation, tumor growth, or a wide variety of other serious diseases. Ovarian tissues are some of the fastest growing tissues in the body, and therefore serve as an excellent model for the study of process that regulate growth, development and differentiation. Because, ovarian dysfunctions cause infertility and other pathological conditions, the study of cellular interactions during growth, development and differentiation of ovarian tissues, as proposed herein, will enhance our understanding of reproductive health, as well as the efficiency of reproduction in animals of agricultural importance.

2001-02337 Functional and Molecular Effects of the Oviduct on Bovine Sperm Capacitation
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Postdoctoral Fellowship; Grant 2001-35203-10983; $90,000; 2 Years

Mammalian sperm must undergo a maturational process called “capacitation” before they are able to fertilize an egg. Capacitation normally occurs in the female reproductive tract or under *in vitro* conditions designed to mimic that environment. The study of sperm capacitation is relevant to increasing the efficiency of assisted reproduction procedures for enhanced production in agricultural species, the preservation of endangered animal species, and the development of new methods for fertility evaluation and contraception. The goal of this project is to increase our understanding of sperm capacitation at the molecular level and determine the effects of the female reproductive tract on this process. In order to study the effect of female reproductive tract components on bovine sperm capacitation, I will use microscopic vesicles generated from the cells lining the oviduct, where capacitation and fertilization normally occur, and oviductal fluid for *in vitro* capacitation studies. The first aim of this proposal is to examine the effects of these female reproductive tract components on sperm viability, capacitation, and the molecular events that occur during capacitation. The second aim of this proposal is to explore the significance of a specific molecular change, called protein tyrosine phosphorylation, that accompanies capacitation. Because protein tyrosine phosphorylation is an important regulator of cellular function in other systems, we believe that this change may result in important changes in protein activity that allow sperm capacitation to occur. A protein that undergoes tyrosine phosphorylation during capacitation will be isolated, cloned, and its activities in the phosphorylated and non-phosphorylated states will be compared.

2001-00769 Calcium Signaling during Embryonic Development in Cattle
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Seed Grant; Grant 2001-35208-10841; $65,000; 2 Years

Emerging technology indicates that it may be now possible to produce commercially large

numbers of bovine embryos by *in vitro* techniques. *In vitro* production of embryos, as well as reproductive performance of dairy and beef cattle, is impaired by early embryonic mortality that may result directly from the excessive induction of programmed cell death (apoptosis). Our studies indicate that the ubiquitous cellular signal, calcium ions, can induce apoptosis. The objective of this research project is to determine cellular and molecular mechanisms by which calcium regulates early embryonic development in cattle. Using bovine embryos produced in culture, we will investigate how cellular calcium is regulated during early development, as well as determine the role of calcium signals in programmed cell death. Such a novel approach seems particularly productive because interaction of the calcium and apoptosis signaling pathways may determine fate of the embryo. Our long-term goal is to increase efficiency of reproductive performance and decrease embryonic mortality in cattle by preventing dysregulation of calcium signaling occurring in the early developmental period. Our experimental methods will include high-resolution digital imaging of live embryos loaded with fluorescent probes for detection of calcium, calcium targets and cell death. The results of this research project will help us to understand mechanisms of calcium signaling during early embryonic development and may lead to development of new cellular and molecular strategies, based on control of cellular calcium, for increasing reproductive efficiency in agriculturally important animals.

2001-02259 Role of Endometrial Glands in Uterine Function

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Grant 2001-35203-10700; $260,000; 3 Years

Infertility, pregnancy loss and intrauterine growth retardation are common problems that affect reproductive efficiency, health and development of livestock, as well as humans. A large percentage of these problems may be attributed to inability of the uterus to support pregnancy. All mammalian uteri have endometrial glands that secrete a variety of substances hypothesized to support development of the conceptus (embryo and associated placental membranes) during pregnancy. This research program has utilized sheep that lack glands in the uterus or the uterine gland knockout (UGKO) model. In this unique UKGO ewe, postnatal growth of endometrial glands is impaired in the neonate, resulting in the absence of glands in the adult. UGKO ewes exhibit defects in the estrous cycle and in establishment and maintenance of pregnancy. Previously, NRICGP-funded research demonstrated that UGKO ewes are unable to maintain peri-implantation conceptus survival and development, indicating that genes expressed by endometrial glands are required for successful maternal support of conceptus growth and development. Objectives of the current studies are to discover genes important for conceptus survival and development using UGKO ewes and modern genomics techniques. Genes that are discovered and functionally characterized will be useful cellular and molecular markers of endometrial differentiation/function and uterine receptivity. The long-term goal of this research is to increase knowledge of uterine gland function in an effort to optimize the reproductive performance and efficiency of animal production agriculture. Knowledge gained from the proposed studies will be useful to design management, biotechnological and genetic applications aimed at enhancing production efficiency.

2001-02166 Placental Nitric oxide and Polyamine Syntheses in Pigs

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Grant 2001-35203-11247; $200,000; 3 Years

The placenta undergoes rapid formation of new blood vessels (angiogenesis) and marked growth during days 20 to 60 of gestation in pigs. The early placental growth is a critical factor for controlling the survival, growth and development of fetal pigs. Nitric oxide and polyamines, which are produced from arginine and ornithine (amino acids or nutrients), are key regulators of placental angiogenesis and growth, as well as embryonic development. Nitric oxide also plays an important role in regulating vascular tone of uterine and placental-fetal vessels, and thus nutrient supplies from maternal to fetal blood. We

hypothesized that: (1) proline (an amino acid or nutrient) is the major source of the ornithine for placental polyamine synthesis, and (2) progesterone and estrogen (reproductive hormones) play a crucial role in regulating placental nitric oxide and polyamine syntheses during early gestation. These novel hypotheses will be tested in this research, with the following specific aims: (1) determine the effects of progesterone and estrogen on nitric oxide synthesis from arginine in porcine placenta during early gestation, and (2) determine the effects of progesterone and estrogen on polyamine synthesis from proline in porcine placenta during early gestation. Because there is still 20 to 50% prenatal mortality in pigs, and because placental insufficiency is a major factor contributing to low birth weights of piglets (0.9 - 1.1 kg) whose postnatal mortality is particularly high (20 to 56%), new knowledge of placental nitric oxide and polyamine syntheses will be beneficial for designing novel nutritional and hormonal interventions to improve the reproductive efficiency of pigs in the U.S. pork industry.

2001-02264 Neuroendocrine Basis of Seasonal Breeding in Sheep

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Grant 2001-35203-10862; $250,000, 3 Years

The long-term goal of this research is to understand the structural and functional changes in the brain that are responsible for the reversible suppression of ovarian function during seasonal anestrus in ewes. It is now clear that infertility during anestrus is caused by a seasonal shift in the response to inhibitory actions of the ovarian hormone, estradiol. We recently have identified neurons located in a specific area of the brain that mediate these actions of estradiol during anestrus, but do not respond to estradiol during the breeding season. We will test two hypotheses for the seasonal difference in these neurons: (1) there is an increase in estrogen receptors in these neurons during anestrus, and (2) there is an increase in neurotransmitter levels of these neurons occurs during anestrus. The results of these studies will provide basic information on the mechanisms suppressing ovarian function in anestrus and may lead to the development of novel approaches to overcome infertility that occurs annually in most breeds of sheep. The information obtained may also provide new insights into the causes of infertility in other domestic species.

2001-02268 Influence of IGF-1 on GnRH Release

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Standard Strengthening Award; Grant 2001-35203-10835; $170,000, 2 Years

Maximal reproductive efficiency depends upon the timely achievement of puberty, estrous cycle expression, and postpartum return to fertility. Malnutrition negatively influences these various aspects of fertility and thus, economically impacts livestock producers. The mechanisms whereby nutrition affects reproduction are not completely understood, but undoubtedly involve the regulation of gonadotropin-releasing hormone (GnRH) from the brain. GnRH stimulates the release of luteinizing hormone (LH) from the pituitary gland that is necessary for ovulation. Impaired fertility during undernutrition is associated with reduced levels of insulin-like growth factor-1 (IGF-1) and IGF-1 influences LH secretion and gonadal function. Thus, IGF-1 may be an important blood-borne link between nutrition and reproduction. The purpose of this grant is to determine if IGF-1 stimulates GnRH release in ewes. The goal of Experiment 1 is to develop an appropriate model wherein the predicted stimulatory effects of IGF-1 on GnRH release can be evaluated. Yearling ewes will be used to determine the period of food restriction necessary to reduce circulating IGF-1 and LH (and thus, GnRH) levels. This animal model will be used in Experiment 2 to determine if IGF-1 treatment stimulates GnRH release during undernutrition. Because GnRH release can be measured directly only in sheep, these findings will provide unique insight into the mechanisms whereby nutrition impairs reproduction. Potentially, these findings could lead to strategies whereby time to puberty or the postpartum period of infertility could be shortened, both of which would have tremendous economic benefit to livestock producers.

2001-02331 Investigation of the Benefits of Early Gestational IGF-1 in Sheep
Wilson, M.E.
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Postdoctoral Fellowship; Grant 2001-35203-10982; $88,726; 2 Years

The long term objective of this research is to devise methods to optimize lamb survival. Existing evidence supports the suggestion that as many as 5% of lambs born die as a result of complications associated with a low birth weight. This proposal focuses on the potential role of growth factors to modulate very early embryonic growth in order to optimize birth weight at term. Towards this end, a better understanding of mechanisms involved in regulating very early embryonic growth and how altering such growth impacts later placental, fetal and even neonatal growth is necessary. This grant addresses that goal of better understanding with three specific aims including: (1) determining the role of systemic hormone treatment on uterine luminal growth factor content during very early gestation; (2) determining how exposure to increased amounts of growth factors during very early gestation alters early embryonic growth, and (3) determining how exposure to increased amounts of growth factors during very early gestation alter fetal and placental development at critical stages of gestation. The objectives of the studies in this grant are to investigate whether increased uterine luminal growth factor content affects proliferation of embryonic cell types and conceptus growth prior to placentome development, through mid-gestation, near term and at birth.

2001-02160 Annual Meeting of the Society for the Study of Reproduction
Sanborn, B.M.
Society for the Study of Reproduction; Madison, WI 53711-2063
Conference Grant; Grant 2001-35203-11206; $10,000; 7 Months

This grant supports the annual meeting of the Society for the Study of Reproduction (SSR) from July 28 through August 1, 2001, in Ottawa, Canada. The SSR represents a unique combination of basic scientists working in experimental systems, domestic animal species, endangered species, primates, and humans. The annual meeting is the major forum for the interchange of ideas that can be directly applied to reproduction and breeding of agriculturally important domestic animal species. Understanding and enhancing livestock breeding and genetic characteristics are important goals for many of our members. In planning SSR 2001, we recognized that this new century will bring striking developments as a result of the new information emerging from genome sequencing initiatives and the advances in developmental biology and cloning. The challenge will be to bring this wealth of knowledge to bear on issues of human health, food production, and the environment. The Keynote Address on the politics of funding, the President's Symposium on "Frontiers in Intracellular Communication", the State-of-the-Art Lectures and minisymposia, and the platform and poster sessions are all designed to help the membership remain abreast of advances and be equipped to meet these challenges. Approximately 40% of attendees at the annual meetings are students; a competition for trainee poster and oral presentations recognizes the contributions of these young scientists. The entire scientific program has been assembled to be of interest and direct relevance to scientists working to improve the efficiency of livestock reproduction. Funds will support the program and trainee travel.

2001-02267 Role of Matrix Metalloproteinase-2 in the Folliculo-Luteal Transition
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Grant 2001-35203-10838; $150,000; 2 Years

The connective tissue matrix of the wall of periovulatory ovarian follicles is degraded and remodeled during ovulation and formation of the corpus luteum. Matrix metalloproteinase-2 (MMP-2) belongs to a family of enzymes that cleave extracellular proteins; its primary substrate is the type IV collagen of basement membranes. Transcriptional expression of MMP-2 in periovulatory ovine follicles

is up-regulated by tumor necrosis factor (TNF). It is proposed that the stimulatory effect of TNF on MMP-2 is mediated by the tumor suppressor/nuclear transcription factor p53. Preliminary studies substantiate that ovulation in ewes is blocked by intrafollicular injection of MMP-2 antibodies; luteinized unruptured follicles were deficient in collagenous/vascularized trabeculae and produced less progesterone than their control luteal counterparts. Accordingly, the basic premise of this grant is that the TNF-p53 system, via MMP-2 induction, contributes to the reorganization of an ovulatory follicle into a fully-competent corpus luteum. Specific objectives using the sheep as an experimental model are to: (1) characterize the metamorphic and endocrine implications of anovulation caused by immunoneutralization of follicular MMP-2; (2) determine the spatial tissue dynamics of p53 and MMP-2 throughout the periovulatory period; (3) clone and sequence the gene encoding MMP-2; and resolve the intermediary role of p53 in the signal transduction mechanism of TNF-activated MMP-2 production. A fundamental understanding of the biomechanics of ovulation and luteal development is essential to the formulation of strategies to manipulate fertility and the establishment of pregnancy.